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Energy and nutrient utilisation of broilers fed soybean meal from two different Brazilian production areas with an exogenous protease

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ABSTRACT

A study was conducted to evaluate the effects of an exogenous protease added to diets having soybean meal (SBM) from two geographic areas in Brazil (South or North). Evaluation was conducted on energy utilisation and nutrient digestibility using 224 slow feathering Cobb × Cobb 500 male broilers. Birds were fed a common starter diet to d 15. Semi-purified experimental diets containing 555 g/kg SBM and 408 g/kg maize starch plus minerals and vitamins were provided afterwards until 24 d. Diets had 0.1 g/kg phytase and 10 g/kg Celite (indigestible marker). Birds were randomly distributed in a 2 × 2 factorial arrangement of the two SBM with or without protease supplementations (0 or 15,000 protease units/kg) from 17 to 24 d. Excreta were collected from 21 to 23 d and ileal content was collected from all birds. Samples of feed, excreta, and ileal content were analysed for determination of total tract retention and ileal apparent digestibility. No interactions between SBM and protease were observed. Broilers fed semi-purified diets formulated with South SBM had lower ($P < 0.05$) ileal digestibility of crude protein and also from most amino acids (AA) when compared with birds fed North SBM. Ileal digestible energy was increased ($P < 0.01$) by 0.51 MJ/kg when birds were fed the diet supplemented with protease. Indispensable and dispensable AA digestibility also increased ($P < 0.05$) with protease supplementation. Ileal digestibility coefficients of Met, Lys, Thr and Val were increased ($P < 0.05$) by 3.1%, 3.1%, 4.8%, and 3.3%, respectively when birds were fed the diet supplemented with protease. Results from this experiment show that utilisation of energy and AA from SBM depends on its origin, but protease supplementation improved their utilisation regardless of the SBM source.

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1. Introduction

Soybean meal (SBM) is the main protein ingredient in poultry feeds. Some tables of ingredient composition (NRC, 1994; FEDNA, 2010; Rostagno et al., 2011) generally provide nutrient profiles for this ingredient based on its crude protein (CP)

Abbreviations: AA, amino acid; AME, apparent metabolisable energy; AME_n, apparent metabolisable energy corrected for zero nitrogen retention; BW, body weight; Ca, calcium; CP, crude protein; d, days; dig., digestible; DM, dry matter; GIT, gastrointestinal tract; IDE, ileal digestible energy; NSP, non-starch polysaccharides; nPP, non-phytate phosphorus; N, nitrogen; PROT, protease units; SBM, soybean meal.

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content. Amino acid (AA) profile and nitrogen (N) digestibility reported in these publications are similar for SBM when CP is taken in account, but none of them recognize the influence of soy genotype, processing conditions, and area of origin on chemical composition (Serrano et al., 2012).

It is usual to consider a linear ratio between digestible AA and CP contents for feed formulation; however, the AA profile, the quality of CP fraction and the nutritive value of SBM might not be as uniform and predictable as it is believed (Irish and Balnave, 1993; Dudley-Cash, 1997). In this context, it has been demonstrated that several factors might affect the chemical composition of SBM. Between these, soy genotype (Cromwell et al., 1999; Palacios et al., 2004), soil type, latitude, location and environmental conditions (van Kempen et al., 2002; Goldflus et al., 2006; Thakur and Hurburgh, 2007), source or country of origin (Karr-Lilienthal et al., 2004; de Coca-Sinova et al., 2008), amount of antinutritional factors, oligosaccharides and non-starch polysaccharides (NSP), and conditions applied during heat-processing of soybean meal (Waldroup et al., 1985; Parsons et al., 1991) have been previously reported. Therefore, information about the different composition of SBM used in feed formulations and, consequently, understand the effects of this difference to improve energy and nutrient utilisation for broiler chickens.

Soybean meal is considered highly digestible for poultry, but there are still possibilities to improve its nutritional value. Studies have reported that the endogenous proteases synthesized and released in the gastrointestinal tract (GIT) may be sufficient to optimize feed protein utilisation (Le Heurou-Luron et al., 1993; Nir et al., 1993). On the other hand, the digestibility of CP and AA in poultry diets has indicated that valuable amounts of protein pass through the GIT without being completely digested (Parsons et al., 1997; Wang and Parsons, 1998; Lemme et al., 2004), then exogenous proteases represents an opportunity to improve protein and AA digestibilities (Bedford, 1996; Angel et al., 2011). Finally, has been known that crude protein and AA digestibility are considerably variable among SBM sources as already mentioned above; therefore, understand the role of exogenous proteases in soybean meals with different compositions has also become important. The objective of the present study was to evaluate the effects of semi-purified diets using two different sources of soybean meal, supplemented or not with an exogenous protease on energy utilisation and nutrient digestibility in broiler chickens.

2. Materials and methods

All procedures used in this study were approved by the Ethics and Research Committee of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

2.1. Birds and housing

A total of 224 one-day-old, slow-feathering Cobb × Cobb 500 male broiler chicks, vaccinated for Marek's disease at the hatchery, with an average BW (body weight) of 45 ± 1.1 g were randomly placed in 48 wire cages ($0.9 \times 0.4\,m^2$). Each cage was equipped with one feeder and one drinker. Birds had *ad libitum* access to water and mash feeds. Average temperature was 32 °C at placement being reduced by 1 °C every 2 d until 23 °C to provide comfort throughout the study. Lighting was continuous until 24 d of age.

2.2. Experimental diets

Birds were allocated to 4 experimental diets with 8 replications of 7 birds each in a completely randomised design. Analysed chemical composition of SBM samples from two main production areas of soy in Brazil were used in this study (Table 1). Soil and environmental conditions are different between these two Brazilian areas, consequently soy growing and amounts of antinutritional factors, oligosaccharides and NSP also can be different. A standard maize-SBM-based broiler starter diet was fed from 1 to 16 d (12.75 MJ/kg AME_n, 217 g/kg CP, 10.5 g/kg Ca, and 5.3 g/kg non-phytate P). Semi-purified experimental diets were provided afterwards to 24 days in a 2 × 2 factorial arrangement of 2 SBM sources (South or North) supplemented or not with protease (Table 2).

The protease utilized in the present study had 15,000 protease units (PROT) per kg of diet (Ronozyme ProAct; Novozymes A/S, Bagsvaerd, Denmark). This was a commercial enzyme product produced by submerged fermentation of *Bacillus licheniformis* containing transcribed genes from *Nocardiopsis prasina* and contained 75,000 PROT/g. One PROT is defined as the amount of enzyme that releases 1 μmol of *p*-nitroaniline from 1 μM of substrate (Suc-Ala-Ala-Pro-Phe-*p*-nitroaniline) per minute at pH 9.0 and 37 °C. All experimental diets had 10 g/kg Celite as indigestible marker (Celite, Celite Corp., Lompoc, CA).

2.3. Experimental procedures and chemical analysis

Excreta were collected twice daily on wax paper from 21 to 23 d being immediately mixed and pooled by cage and stored at −20 °C until analysis. Previous to calorimetry, excreta were dried in a forced air oven at 55 °C (DeLeo, Porto Alegre, Brazil) and ground to pass through a 0.5-mm screen. Ileal content was collected from all birds at 24 d after electrical stunning using 45 V for 3 s. Ileal content was collected from the Meckel's diverticulum to approximately 2 cm cranial to the ileo-caecal junction. The procedure was done by flushing digesta with distilled water into plastic containers, pooled by cage,

Table 1
Analysed gross energy and chemical composition of soybean meal (on DM basis).[a]

Item	South source	North source
Gross energy, MJ/kg	16.5	16.6
Crude protein (N × 6.25), g/kg	447.9	467.5
Ether extract, g/kg	39.2	15.8
Crude fibre, g/kg	49.8	39.2
Ash, g/kg	54.6	52.0
Calcium, g/kg	3.4	4.1
Phosphorus, g/kg	6.7	6.2
Total amino acid, g/kg		
Lysine	29.0	29.5
Methionine	5.8	6.1
Cysteine	7.1	7.5
Threonine	18.0	18.5
Valine	22.2	23.1
Tryptophan	6.1	6.3
Arginine	32.4	32.6
Isoleucine	21.0	21.4
Leucine	34.5	35.5
Histidine	12.6	12.9
Phenylalanine	23.9	24.5

[a] Values presented are for the means of duplicate analyses for all components.

Table 2
Ingredient composition of the semi-purified experimental diets fed from 17 to 24 d.

Ingredients	Inclusion, g/kg
Soybean meal	555.1
Maize starch	407.7
Dicalcium phosphate	9.6
Limestone	10.6
Salt	5.1
Vitamin and mineral premix[a]	1.5
Celite[b]	10.0
Phytase[c]	0.1

[a] Composition per kg of feed: vitamin A, 8000 UI; vitamin D_3, 2000 UI; vitamin E, 30 UI; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg.
[b] Indigestible marker (Celite, Celite Corp., Lompoc, CA).
[c] Ronozyme HiPhos with 10,000 fungal phytase units/g (Novozymes A/S, Bagsvaerd, Denmark).

immediately frozen in liquid nitrogen, and stored in a freezer at −20 °C until lyophilised (Christ Alpha 2-4 LD Freeze Dryer, Newtown, UK).

Semi-purified diet and freeze-dried samples of ileal contents were ground to pass through a 0.5-mm screen in a grinder (Tecnal, TE-631/2, São Paulo, Brazil). Dry matter (DM) analysis of samples was performed after oven drying the samples at 105 °C for 16 h (method 934.01; AOAC International, 2006). Ileal contents, excreta, and diet samples were analysed for gross energy using a calorimeter calibrated with benzoic acid as a standard (IKA Werke, Parr Instruments, Staufen, Germany). Calculations of ileal digestible energy (IDE), AME (apparent metabolisable energy), and AME_n (apparent metabolisable energy corrected for zero nitrogen retention) were done afterwards. Crude protein (N × 6.25) was determined by combustion method (method 968.06; AOAC International, 2006). The calculated AME was corrected to zero N retention (AME_n) using a factor of 0.034 MJ/g (Hill and Anderson, 1958). Acid insoluble ash concentration in the diets, excreta, and ileum samples were determined using the method described by Vogtmann et al. (1975), and Choct and Annison (1992). Dietary and ileal digesta samples were analysed for CP and AA at the University of Missouri Experiment Station Chemical Laboratories, Columbia [method 982.30 E (a, b, c), AOAC International, 2006].

2.4. Calculations

Apparent ileal digestibility, total tract utilisation and AME_n were calculated using the following equations as described by Kong and Adeola (2014):

$$\text{Digestibility}(\%) = [1 - (M_i/M_o) \times (E_o/E_i)] \times 100,$$

$$AME_n(\text{MJ/kg}) = GE_i - [GE_o \times (M_i/M_o)] - 8.22 \times \{N_i - [N_o \times M_i/M_o)]\},$$

Table 3
Coefficients of ileal digestibility and total tract retention of dry matter (DM), crude protein, and energy by broilers fed soybean meal-based diets from two regions and supplemented or not with protease.[a]

Item	Ileal digestibility coefficient			Total tract retention coefficient			
	DM	IDE[b], MJ/kg DM	Crude protein	DM	AME, MJ/kg DM	AME_n, MJ/kg DM	Crude protein
SBM source[c]							
South	0.673	13.11	0.829	0.682	12.95	12.36	0.454
North	0.688	13.30	0.849	0.691	13.06	12.40	0.460
Protease, PROT/kg[d]							
0	0.664	12.95	0.828	0.676	12.79	12.30	0.442
15,000	0.696	13.46	0.850	0.699	13.22	12.46	0.473
SEM	0.035	0.543	0.021	0.030	0.472	0.213	0.036
Main effect *P*-value							
SBM	0.196	0.274	0.004	0.378	0.486	0.647	0.628
Protease	0.007	0.006	0.001	0.035	0.001	0.044	0.019
SBM × protease	0.884	0.325	0.712	0.811	0.847	0.565	0.915

[c]Soybean meal from South or North regions in Brazil.
[a] Means were obtained from 8 replicate cages of 7 birds each.
[b] IDE, ileal digestible energy.
[d] PROT, protease units per kg of feed.

where M_i is the concentration of acid insoluble ash in the diet in grams per kilogram of DM; M_o is the concentration of acid insoluble ash in the excreta and ileal digesta in grams per kilogram of DM output; E_i is the concentration of DM, CP, energy, or AA in the diet in milligrams per kilogram of DM; and E_o is the concentration of DM, CP, energy, or AA in the excreta and ileal digesta in milligrams per kilogram of DM. GE_i is gross energy (MJ/kg) in the diet; GE_o is the gross energy (MJ/kg) in the excreta; N_i is nitrogen concentration in the diet, and N_o is nitrogen concentration in the excreta in g/kg DM.

2.5. Statistical analysis

The experiment was conducted in a completely randomized factorial arrangement of 2 SBM sources (South or North) × 2 protease supplementations (without or with protease). Data were submitted to a 2-way ANOVA using the GLM procedure of SAS Institute (SAS, 2009). Significance was accepted at $P < 0.05$ and mean differences were separated using Tukey's HSD test (Tukey, 1991).

3. Results

Analysis of protease in the experimental diets indicated that the supplemental enzyme had activity well proportioned with the expected value (formulated 15,000 PROT/kg and analysed 16,067 PROT/kg). Effects of SBM source and protease supplementation on total tract retention and apparent ileal digestibility are shown in Table 3. Only the main effects are presented in the tables, as SBM x protease interactions were not significant ($P > 0.05$) for any of the tested parameters. There were no interactions between SBM source and protease supplementation for ileal digestibility and total tract retention in 24-d-old broilers. There were no effects of SBM source on IDE, AME, and AME_n; however, the ileal digestibility of CP was 2.4% higher for SBM from North region ($P < 0.05$).

Differences in AME and IDE were found between diets without and with exogenous protease ($P < 0.01$) with values of 12.79 MJ/kg and 13.22 MJ/kg for semi-purified diets without protease and 13.22 MJ/kg and 13.46 MJ/kg with protease, respectively. In the present study, AME and IDE were improved ($P < 0.01$) by 0.43 and 0.51 MJ/kg, respectively when protease was supplemented.

The coefficients of digestibility of indispensable AA for broilers fed SBM from different sources and protease supplementation are presented in Table 4. No interactions between SBM source and protease were observed on AA digestibility in 24-d-old broilers. Apparent ileal digestibility coefficients of total AA and most of indispensable AA were higher ($P < 0.05$) for SBM from North source. Digestibility of Arg, Lys, Leu, Thr, and Met increased ($P < 0.05$) by 0.7%, 1.0%, 2.0%, 3.9%, and 2.4% when broilers were fed SBM from the North. Additionally, ileal digestibility coefficients of Met, Lys, Thr, Val, and Arg were increased ($P < 0.001$) by 3.1%, 3.1%, 4.8%, 3.3%, and 1.9% respectively when birds were fed the diet supplemented with protease.

Apparent ileal digestibility coefficients of all dispensable AA were higher ($P < 0.05$) in broilers fed semi-purified diets supplemented with protease (Table 5). The digestibility of Ala, Cys, Gly, and Ser increased ($P < 0.001$) by 2.5%, 6.8%, 3.9%, and 3.8% when broilers were fed soy-based diets supplemented with protease. Soybean meal from North source had higher ($P < 0.05$) digestibility coefficients of Ala, Hyp, Ser, and Tyr when compared to the SBM from South.

Table 4
Apparent ileal digestibility coefficient of indispensable amino acids using SBM from two regions and supplemented or not with protease in broiler chickens.[a]

Item	Total AA[b]	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
SBM source[c]											
South	0.844	0.909	0.873	0.850	0.850	0.876	0.881	0.856	0.786	0.872	0.826
North	0.858	0.915	0.878	0.868	0.867	0.885	0.903	0.874	0.818	0.891	0.844
Protease, PROT/kg[d]											
0	0.836	0.903	0.861	0.845	0.845	0.867	0.878	0.851	0.778	0.869	0.821
15,000	0.866	0.921	0.889	0.873	0.872	0.895	0.906	0.879	0.817	0.894	0.849
SEM	0.025	0.017	0.020	0.023	0.024	0.020	0.022	0.022	0.029	0.021	0.026
Main effect *P*-value											
SBM	0.004	0.201	0.321	0.004	0.013	0.086	0.001	0.005	0.002	0.001	0.026
Protease	0.002	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.009
SBM × protease	0.132	0.317	0.634	0.267	0.248	0.248	0.670	0.535	0.147	0.192	0.425

[a] Means were obtained from 8 replicate cages of 7 birds each.
[b] Average of all amino acids.
[c] Soybean meal (SBM) from South or North regions of Brazil.
[d] PROT, protease units per kg of feed.

Table 5
Apparent ileal digestibility coefficient of dispensable amino acids using SBM from two regions and supplemented or not with protease in broiler chickens.[a]

Item	Ala	Asp	Cys	Glu	Gly	Hyl	Hyp	Orn	Pro	Ser	Tyr
SBM source[b]											
South	0.836	0.832	0.714	0.892	0.800	0.801	0.397	0.790	0.845	0.819	0.856
North	0.852	0.833	0.728	0.898	0.807	0.813	0.496	0.821	0.851	0.843	0.862
Protease, PROT/kg											
0	0.828	0.819	0.695	0.886	0.788	0.782	0.408	0.740	0.834	0.815	0.848
15,000	0.849	0.846	0.746	0.903	0.820	0.833	0.485	0.871	0.863	0.847	0.875
SEM	0.025	0.021	0.039	0.017	0.026	0.098	0.058	0.083	0.022	0.028	0.019
Main effect *P*-value											
SBM	0.023	0.956	0.222	0.271	0.397	0.519	0.001	0.067	0.308	0.002	0.035
Protease	0.001	0.001	0.001	0.002	0.001	0.013	0.003	0.001	0.001	0.001	0.001
SBM × protease	0.626	0.577	0.817	0.705	0.993	0.626	0.001	0.052	0.549	0.525	0.460

[a] Means were obtained from 8 replicate cages of 7 birds each.
[b] Soybean meal (SBM) from South or North regions of Brazil.

4. Discussion

Improvements in AME_n for broilers fed SBM-based diets from different sources is likely to be attributed to differences in carbohydrate composition. Usually, SBM have approximately 10% free sugars (Choct et al., 2010), 6% soluble NSP, 18–21% insoluble NSP, and less than 1% starch (Bach Knudsen, 1997). Studies have demonstrated that SBM nutritive value also depends on the presence of indigestible carbohydrates, in particular, the amount of oligosaccharides (raffinose and stachyose), because endogenous enzymes may have limited access to the nutrients bound in cell walls, limiting energy utilisation and nutrient digestibility (Slominski and Campbell, 1990; Castell et al., 1996; Kocher et al., 2003). Oligosaccharides in SBM can impact energy utilisation also through loss of water in the lumen due to their hydrophilic nature and increase colonization of microbes in the gastrointestinal tract, leading to increased excreta output (Kocher et al., 2003). Concentrations of raffinose and stachyose in conventional SBM have been reported to vary with geographical location, harvest conditions, and post-harvest processing (Parsons et al., 1991; Karr-Lilienthal et al., 2004).

Differences in digestibility of indispensable AA between SBM grown in different areas have to be taken in consideration and it should be expected that those are presented in reference tables in the future. Variability in SBM composition was demonstrated by de Coca-Sinova et al. (2008) who evaluated SBM from six different origins and found considerable variation in chemical composition and protein quality, which caused differences in AA digestibility with 21-d-old broilers. These authors also reported that the SBM with higher levels of CP and lower levels of trypsin inhibitor activity showed higher AA digestibility. Factors such as genotype, processing and environmental conditions during growing and harvesting of beans have been related to affect the chemical composition of SBM (Thakur and Hurburgh, 2007; Frikha et al., 2012).

In the present study, CP and AA digestibilities were higher in broilers fed diets supplemented with 15,000 PROT/kg than birds fed diets without protease. This response is in agreement with findings by Angel et al. (2011) who observed higher CP digestibility using maize-soybean based diets supplemented with increased levels of the same protease utilized in the present study ranging from 7500 to 60,000 PROT/kg in 22-d-old broilers. Angel et al. (2011) also reported that the same mono-component protease product at any concentration increased apparent digestibility of Arg, Ile, Lys, Thr, His, Asp, Cys, and Ser in broiler chickens. Freitas et al. (2011) demonstrated an increase of 1.8% in CP digestibility when protease was added to the high-protein diets (7% more than the recommended CP level), whereas an improvement of 1% was seen in the low-protein diets. Vieira et al. (2013) also found improvements in ileal digestibility of Lys, Cys, Thr, Ile, Asp, and Glu in turkeys fed diets supplemented with 15,000 PROT/kg.

There were no interactions between SBM source and protease supplementation for all evaluated responses. Considering the AA content and nutrient composition of these two SBM sources and taking into account the digestibility coefficients and energy utilisation, it was possible that the protease supplementation on SBM from South had the same result that using North SBM not supplemented with exogenous protease.

The interpretation of results from studies conducted with proteases is difficult in part because of the lack of information about protease characteristics and the confounding effects of more than one enzymatic activity in a single enzyme product. The diversity of feed ingredients also contributes to inconsistent and variable results (Simbaya et al., 1996; Marsman et al., 1997). Studies done with mono-component proteases allow easier interpretation. However, the literature on SBM-based diets having protease is scarce. Many of the adverse responses to exogenous proteases have been reported due to a lack of understanding about enzyme effect, creating nutrient imbalances, and then a true failure of the enzyme potential of modify its substrate (Marsman et al., 1997; Cowieson et al., 2006; Freitas et al., 2011). Thus, it is critical that knowledge of ingredient quality and the response to supplemented protease is known in advance so that diets may be formulated strategically to allow for the expected improvements on bird retention of nutrients (Cowieson et al., 2006).

Amino acids derived from protein degradation are expected to become available for intestinal absorption, depending, in part, on the affinity between enzyme and substrate. Improvements in AA digestibility derived from any exogenous proteases are also dependent on the protein ingredients used in feed formulation. In conclusion, SBM used in diets supplemented with 15,000 protease units/kg had a beneficial impact on energy utilization and nutrient digestibility for broilers. Finally, it is suggested that the digestibility of CP and most of AA were higher using SBM from the North source due to differences in the indigestible composition.

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